Agilent G1701DA GC/MSD ChemStation

Drug Analysis Software Getting Started

Agilent Technologies

Notices

© Agilent Technologies, Inc. 2005

No part of this manual may be reproduced in any form or by any means (including electronic storage and retrieval or translation into a foreign language) without prior agreement and written consent from Agilent Technologies, Inc. as governed by United States and international copyright laws.

Manual Part Number

G1701-90055

Edition

First edition, June 2005

Printed in USA

Agilent Technologies, Inc.
5301 Stevens Creek Boulevard
Santa Clara, CA 95052

Warranty

The material contained in this document is provided "as is," and is subject to being changed, without notice, in future editions. Further, to the maximum extent permitted by applicable law, Agilent disclaims all warranties, either express or implied, with regard to this manual and any information contained herein, including but not limited to the implied warranties of merchantability and fitness for a particular purpose. Agilent shall not be liable for errors or for incidental or consequential damages in connection with the furnishing, use, or performance of this document or of any information contained herein. Should Agilent and the user have a separate written agreement with warranty terms covering the material in this document that conflict with these terms, the warranty terms in the separate agreement shall control.

Safety Notices

CAUTION

A **CAUTION** notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in damage to the product or loss of important data. Do not proceed beyond a **CAUTION** notice until the indicated conditions are fully understood and met.

WARNING

A WARNING notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a WARNING notice until the indicated conditions are fully understood and met.

GC/MSD ChemStation Quick Reference

Introduction

Overview of the Drug Analysis Software 4
Hardware 5
Online Help 6
Getting Started with the Drug Analysis Software 11

Instrument Control

Instrument Control View 13
Instrument Control Software Menus 17
Tune and Vacuum Control Menus - EI Mode Software Menus 18
Tune and Vacuum Control Menus - CI Mode Software Menus 19

Drug Data Analysis

Drug Data Analysis View 20
Drug Data Analysis Software Menus 26
Parametric Retrieval Software Menus 27
DQEdit Software Menus 28
DrugID Software Menus 29
Review Data Software Menus 30
RTLock Software Menus 31

Common ChemStation Tasks

To Tune your MSD 32
To Run a Sample 34
To Edit a Method 36
To Set up Batches (Sequences) 38
To Analyze MS Data 40
To Use Spectral Libraries 43
To Use Review Data 45
To Update Retention Times Using DrugID 47
To Review Quantitative Results Using DQEdit 49
To Use Retention Time Locking 51

This booklet is an introduction to the Drug Analysis software. Once you become familiar with its contents you can easily explore the software on your own. Comprehensive information on all software features and functions is available in the online help files.

Overview of the Drug Analysis Software

The Drug Analysis software combined with an Agilent GC/MSD forms an integrated drug analysis system. The software includes general drug methods and report formats which can be used as a starting point for performing drug analysis in your lab as well as specialized methods to analyze several drugs regulated by the U.S. National Institute on Drug Abuse (NIDA).

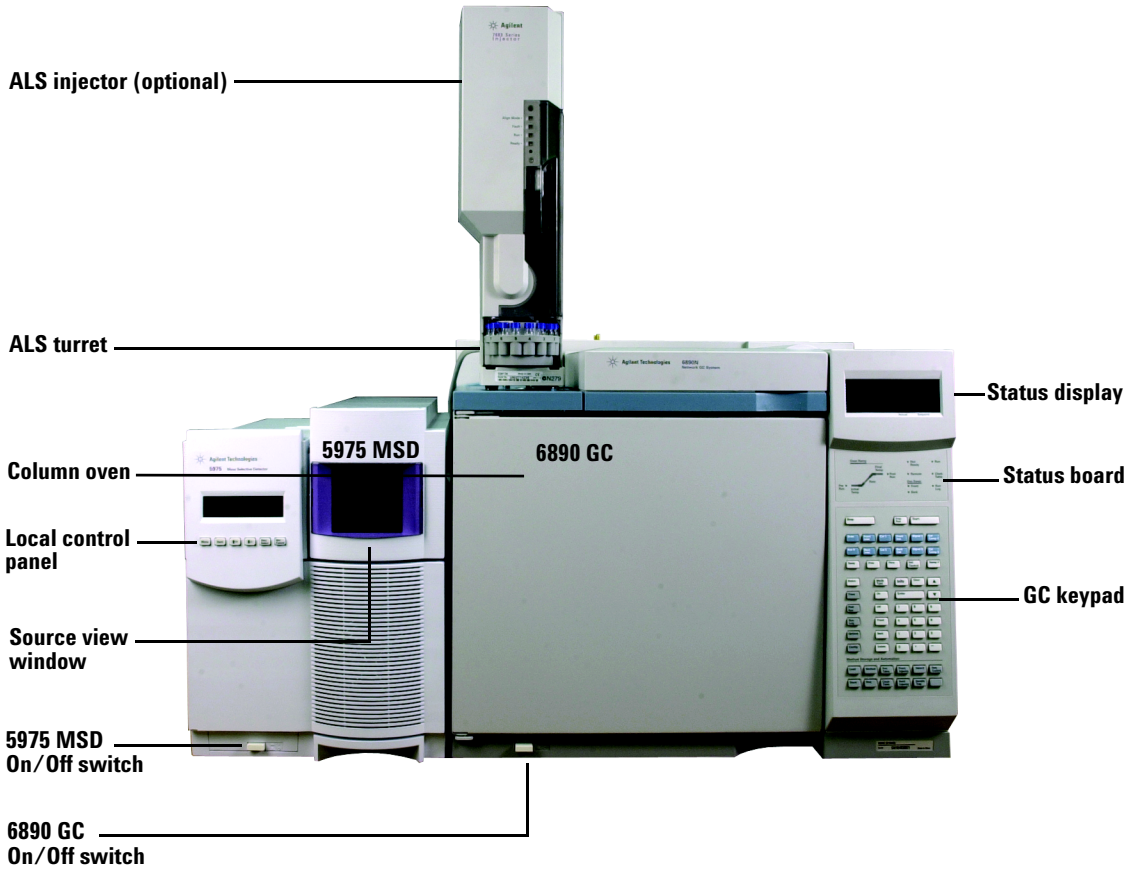
Although most parameters are set at the factory, some, such as GC conditions, must be set to match the particular instrument and application in your laboratory. Additionally, the response and retention times information in the quantitation database will need to be updated so that the compounds will be correctly identified and quantitated on your system.

The Drug Analysis software contains a feature called “Intelligent Sequencing” that lets you define criteria for handling situations that may arise during batch runs. This feature lets you specify what to do if blanks are contaminated, ISTD criteria are not met, analyte concentrations are outside normal limits, and other situations. Some possible actions taken as a result of Intelligent Sequencing are injecting additional blanks, re-injecting samples, and pausing or aborting the batch. Intelligent Sequencing options are stored with the method.

Intelligent Sequencing reduces the number of batches that have to be run. Quality guidelines in the methodologies can be followed without an operator constantly monitoring a run or batch. This is accomplished by saving a method of interest with preset decisions that are carried out depending on the result of each sample. See “Batch Intelligent Sequencing" on page 37 for more details.

Hardware

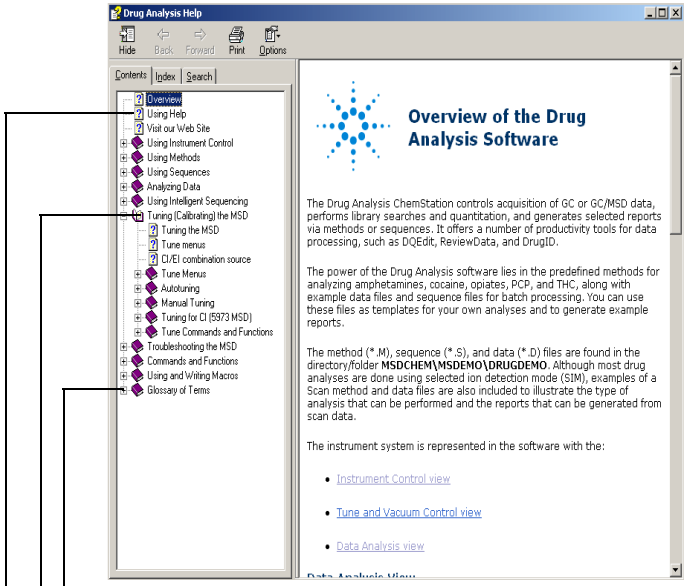
5975 MSD with a 6890 GC



Online Help

Online help files contain extensive information and tutorials about instrument control, data acquisition, data analysis, methods, sequencing, tuning, troubleshooting, and how to use system commands and variables.

To access the online help, select **Help** topics from the Help menu in any window, click on the help icon in the toolbar, or click the help button on any dialog box.


Drug Analysis Help
Hide
Back
Forward
Print
Options
Contents
Index
Search
Overview
Using Help
Visit our Web Site
Using Instrument Control
Using Methods
Using Sequences
Analyzing Data
Using Intelligent Sequencing
Tuning (Calibrating) the MSD
Tuning the MSD
Tune menus
CI/EI combination source
Tune Menus
Autotuning
Manual Tuning
Tuning for CI (5973 MSD)
Tune Commands and Functions
Troubleshooting the MSD
Commands and Functions
Using and Writing Macros
Glossary of Terms
Overview of the Drug Analysis Software
The Drug Analysis ChemStation controls acquisition of GC or GC/MSD data, performs library searches and quantitation, and generates selected reports via methods or sequences. It offers a number of productivity tools for data processing, such as DQEdit, ReviewData, and DrugID.
The power of the Drug Analysis software lies in the predefined methods for analyzing amphetamines, cocaine, opiates, PCP, and THC, along with example data files and sequence files for batch processing. You can use these files as templates for your own analyses and to generate example reports.
The method (*.M), sequence (*.S), and data (*.D) files are found in the directory/folder MSDCHEM\MSDEMO\DRUGDEMO. Although most drug analyses are done using selected ion detection mode (SIM), examples of a Scan method and data files are also included to illustrate the type of analysis that can be performed and the reports that can be generated from scan data.
The instrument system is represented in the software with the:
Instrument Control view
Tune and Vacuum Control view
Data Analysis view

Item	Description
Hide/Show	Lets you turn on or off the display of the list of help topics.
Back	Goes back to the previous help topic.
Print	Lets you print the current book or help topic.
Contents	Displays the list of help topics (shown above).
Index	Lets you use keywords to search the help index for a particular topic.
Search	Lets you type a word or phrase and then displays a list of all the topics in the online help that contain those words.
Options	Lets you change various help options such as the display of tabs.

Help icons

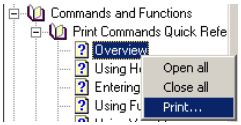
Indicates a book containing more help topics. To open a book, select it then double-click.

Indicates an open book of help topics. To close an open book, select it then double-click.

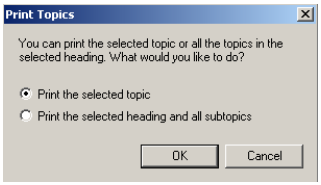
Indicates a help topic. To jump to a help topic, select it then double-click.

To print **a single** help topic:

1 Highlight the topic you want to print, then right-mouse click, and select **Print**....

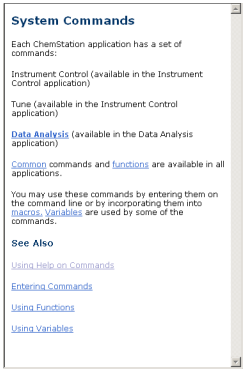


2 Select **Print the selected topic** and click **OK**.


Print Topics
You can print the selected topic or all the topics in the selected heading. What would you like to do?
Print the selected topic
Print the selected heading and all subtopics
OK
Cancel

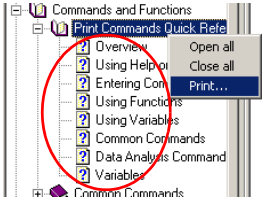
3 Verify the printer selection and click **Print**.

4 The information on that single topic will print. The topics linked to it will not print.

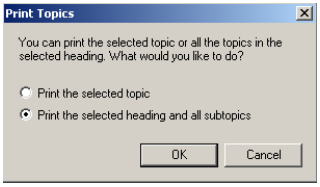

System Commands
Each ChemStation application has a set of commands:
Instrument Control (available in the Instrument Control application)
Tune (available in the Instrument Control application)
Data Analysis (available in the Data Analysis application)
Common commands and functions are available in all applications.
You may use these commands by entering them on the command line or by incorporating them into macros. Variables are used by some of the commands.
See Also
Using Help on Commands
Entering Commands
Using Functions
Using Variables

To print **all subtopics in a heading** at once:

1 Highlight the topic you want to print, then right-mouse click, and select **Print**....

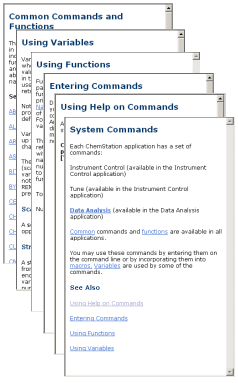


2 Select **Print the selected heading and all subtopics**, and click **OK**.


Print Topics
You can print the selected topic or all the topics in the selected heading. What would you like to do?
Print the selected topic
Print the selected heading and all subtopics
OK
Cancel

3 Verify the printer selection, and click **Print**.

4 The information for ALL topics within the heading of the selected topic will print. In this case, all topics under **Print Commands Quick Reference** would print, which is about 26 pages of information.



Hardware manuals on CD-ROM

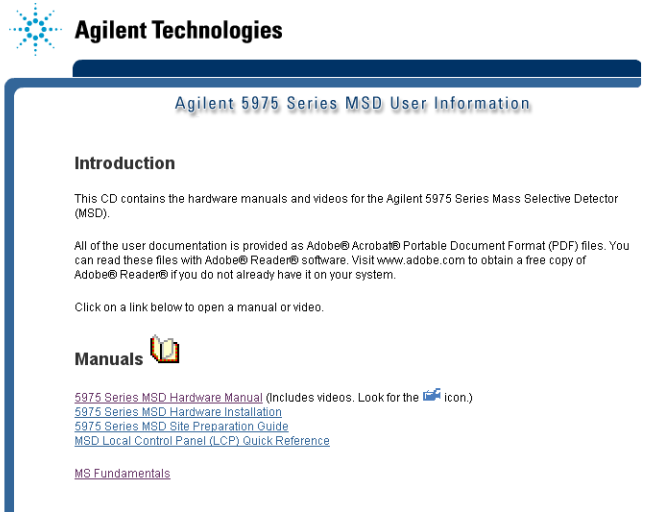
Each piece of hardware is accompanied by a CD-ROM which contains hundreds of pages of reference material as well as videos describing how to operate, maintain, and troubleshoot the equipment.

Using the manuals on CD-ROM

1 The manuals on the CD-ROM are presented in Adobe Acrobat PDF format. The videos are incorporated into the PDF manuals (may require QuickTime), and are also viewable directly from the CD-ROM using Microsoft Media Player.

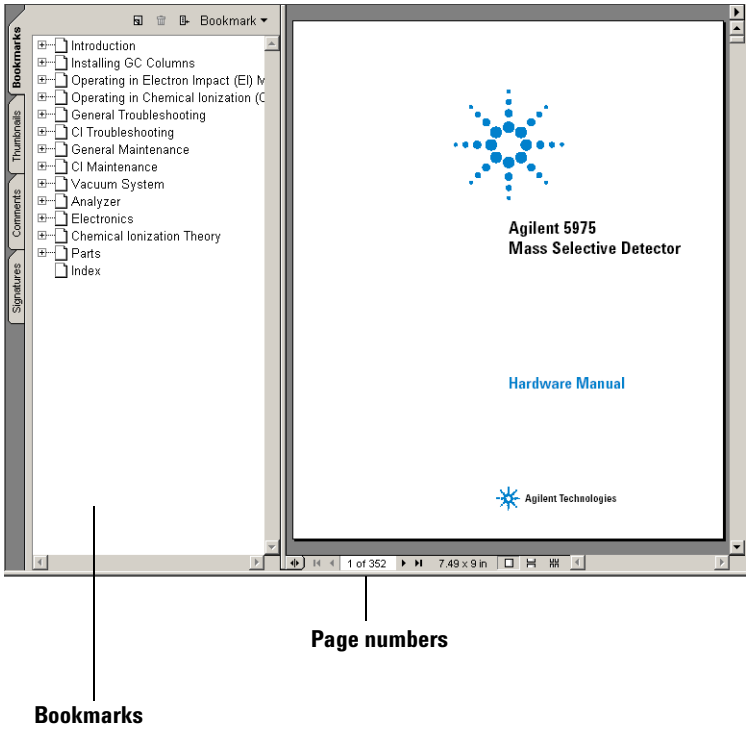
- Access Adobe.com for a free download if you do not have Adobe Acrobat Reader.
- Access Apple.com/quicktime for a free download of QuickTime.

2 Insert the CD-ROM into your disk drive and it will automatically display an opening menu, listing all the books on that CD-ROM, similar to this:


Agilent Technologies
Agilent 5975 Series MSD User Information
Introduction
This CD contains the hardware manuals and videos for the Agilent 5975 Series Mass Selective Detector (MSD).
All of the user documentation is provided as Adobe® Acrobat® Portable Document Format (PDF) files. You can read these files with Adobe® Reader® software. Visit www.adobe.com to obtain a free copy of Adobe® Reader® if you do not already have it on your system.
Click on a link below to open a manual or video.
Manuals
5975 Series MSD Hardware Manual (Includes videos. Look for the icon.)
5975 Series MSD Hardware Installation
5975 Series MSD Site Preparation Guide
MSD Local Control Panel (LCP) Quick Reference
MS Fundamentals

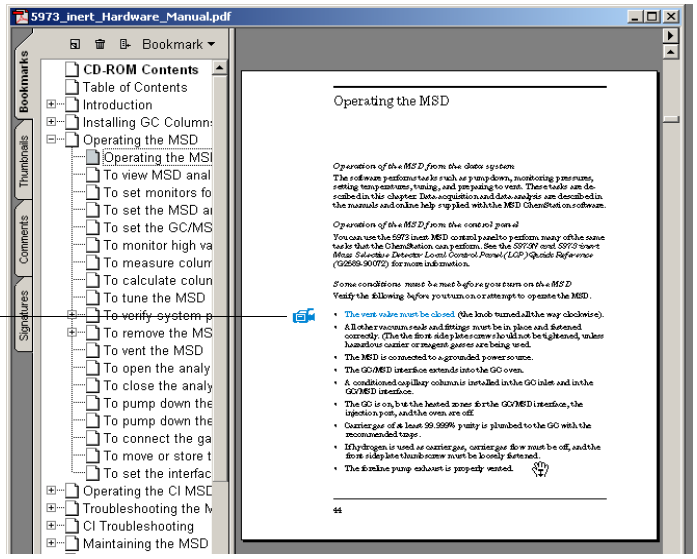
Sample opening menu on a Learning Products CD-ROM

3 Move the cursor over any of the books listed. When the cursor turns into a selection **hand**, click the left mouse button to select the book. The first page of the book and the bookmarks will be displayed.



4 Click any **bookmark** in the left column (such as **Operating the MSD**) and the corresponding page will be displayed.

5 You may print any single page, or group of pages. Select **Print**, then enter the page(s) you want printed, using the page numbers shown at the bottom of the screen.


5973_inert_Hardware_Manual.pdf
Bookmark
Bookmarks
Thumbnails
Comments
Signatures
CD-ROM Contents
Table of Contents
Introduction
Installing GC Column
Operating the MSD
To view MSD anal
To set monitors fo
To set the MSD a
To set the GC/MS
To monitor high va
To measure colum
To calculate colun
To tune the MSD
To verify system p
To remove the MS
To vent the MSD
To open the analy
To close the analy
To pump down the
To pump down the
To connect the ga
To move or store t
To set the interfac
Operating the CI MSD
Troubleshooting the M
CI Troubleshooting
Maintaining the MSD
Operating the MSD
Operation of the MSD from the data system
Operation of the MSD from the control panel
Some conditions must be met before you turn on the MSD
Verify the following before you turn on or attempt to operate the MSD.
The vent valve must be closed (the knob turned all the way clockwise).
The GC/MSD interface extends into the GC oven.
The MSD is connected to a grounded power source.
The GC is on, but the heated zones for the GC/MSD interface, the injection port, and the oven are off.
If hydrogen is used as carrier gas, carrier gas flow must be off, and the front side plate thumbscrew must be loosely fastened.
The foreline pump exhaust is properly vented.

Video icon

6 The **video** icon identifies sections that contain videos on maintenance procedures. Click this icon to view the video clip. The video will stop automatically when finished, or you can press [Escape] to stop it whenever you want.

7 When you move your cursor over a cross reference, it changes to a pointing finger, which indicates the text is linked electronically to the page indicated. Click the cross reference to jump to the indicated page. Right click *to return* to the previous page.

Getting Started with the Drug Analysis Software

Your GC/MSD system is represented in the Drug Analysis software by a set of views.

Instrument Control view

This view lets you edit a method, set up and start a single run or sequence, tune the MSD, and access the Data Analysis software. You can also fine-tune and monitor discrete parts of GC and/or MSD control and display various status monitors.

Drug Data Analysis view

Drug Data Analysis is an offline view that runs independently of the other views. It is used for integrating, quantifying and identifying your data, as well as generating various reports. It also offers various productivity tools such as Review Data, DQEdit and DrugID.

Tune and Vacuum Control

This view is used for calibrating your MSD.

Example Files

The Drug Analysis software ships with several predefined methods for analyzing amphetamines, cocaine, opiates, PCP, and THC, along with example data files and sequence files for batch processing. These files are found in the **msdchem\msdemo\drugdemo** directory. These demo files can used to help you become familiar with the Drug Analysis software.

You can also use these files as templates for your own analyses. To do so, you will need to customize the method parameters based on your particular column and instrument.

AMP.M This method is used for analysis of amphetamine and methamphetamine and deuterated analogs as TFA derivatives from addition of MBTFA. Column used is DB-1701, 10 m 0.18-mm i.d., 0.40-mm film.

COCAINE.M Analysis of benzoylecogonine (BE) and d-3 BE as butyl ester derivative using butyl iodide. Column used is HP-1, 12 m 0.20-mm i.d. polymethylsiloxane, 0.33-mm film thickness.

OPIATE.M Analysis of opiates: morphine, codeine, and their deuterated d-3 analogs as TFA derivatives form addition of MBTFA. Column used is DB-1, 10 m 0.18-mm i.d., 0.4-mm film.

PCP.M Analysis of Phencyclidine (PCP) and deuterated d-5 PCP (internal standard) extracted from alkalinized urine. Column HP-1, 12 m 0.20-mm i.d., polymethylsiloxane.

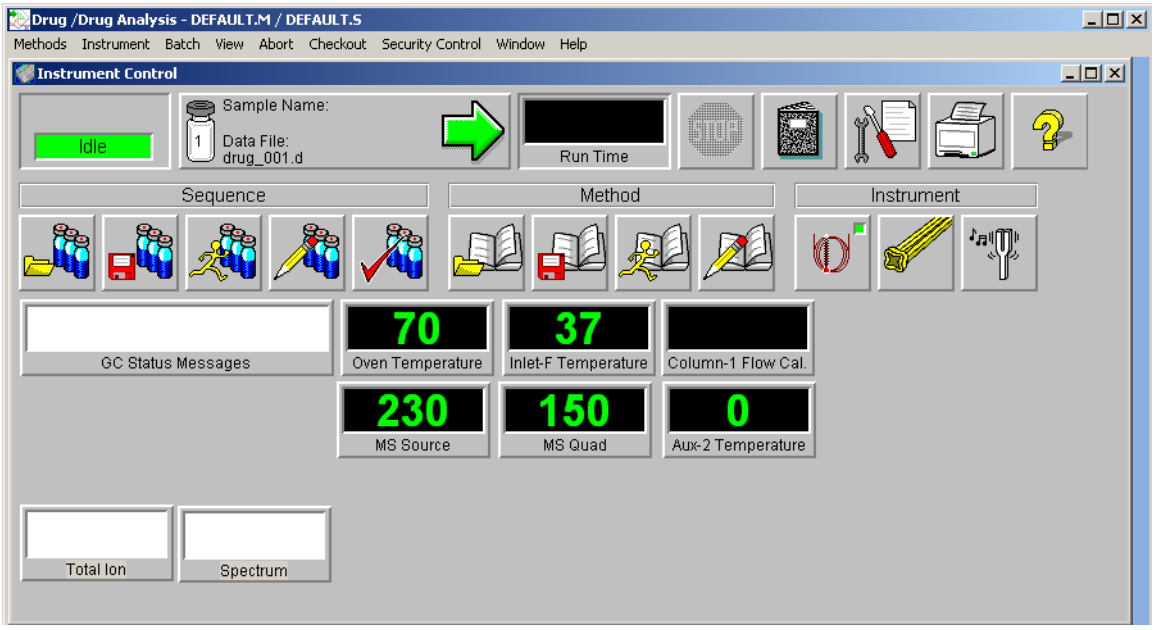
SCANDEF.M Analysis for methadone, cocaine, codeine, monoactylmorphine, and diacetylmorphine in Scan acquisition mode, using caffeine as an internal standard. In this method, the drug report format shows the unknown, first library hit, Text results - Vert. Ion traces, using the Intermediate report type.

THC.M Analysis of THC-COOH and deuterated THC-COOH derivatized with methyl iodide. Column used is DB-1701, 10 m × 0.18-mm i.d., 0.4-mm film.

Instrument Control View

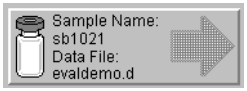
The Instrument Control view is displayed when you start up the GC/MSD ChemStation. This is where you set and monitor instrument parameters, perform an autotune, and start a run. If you are in a different view, select **View/Instrument Control** to access this view.

NOTE See the online help for more details on the menus, buttons, or windows used in the software.



Acquisition Status

Lets you tell at a glance the status of the current instrument or run (if any).



Start

Lets you start a run. When a run is not in progress, the arrow is green. To start a run, click the green arrow, fill in the Sample Information, and click **Start Run**. When a run is in progress, the arrow is gray.

This button also shows the current data file name, sample information (if any), and the vial number (1 if no ALS).

Run Time Clock

Shows the elapsed time since the beginning of the run if a run is in progress. The scheduled run time is shown below the digital clock. When a run is not in progress, the Run Time clock shows the elapsed time since the last run.

Stop

Lets you stop the system when it is in PreRun, Run, or PostRun mode. The stop sign is red when a run is in progress and gray when a run is not in progress.

Logbook

Displays a menu where you can choose to view, open, clear, save, or print a particular logbook.

Maintenance Due

Displays the Select Early Maintenance Feedback (EMF) action dialog box.

Print

Displays a dialog box with such printable items as sequence log, current sequence, instrument parameters, Data Analysis parameters, and detailed Data Analysis parameters.

Help

Displays a menu where you can select an online help topic for the Instrument Control view. Choose Help topics to go to the online help for the entire system.

Load Sequence

Opens the Load Sequence dialog box.

Save Sequence
Opens the Save Sequence dialog box.

Run Sequence
Opens the Start Sequence dialog box.

Edit Sequence
Opens the Sample Log Table dialog box.

Simulate Sequence
Tests a sequence.

Load Method
Opens the Load Method dialog box.

Save Method
Saves the current method.

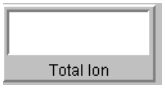
Run Method
Opens the Start Run dialog box.

Edit Method
Lets you edit the current method.

GC Parameters
Lets you edit the current GC parameters and GC monitors.

MS Parameters
Lets you edit the current MS parameters and MS monitors.

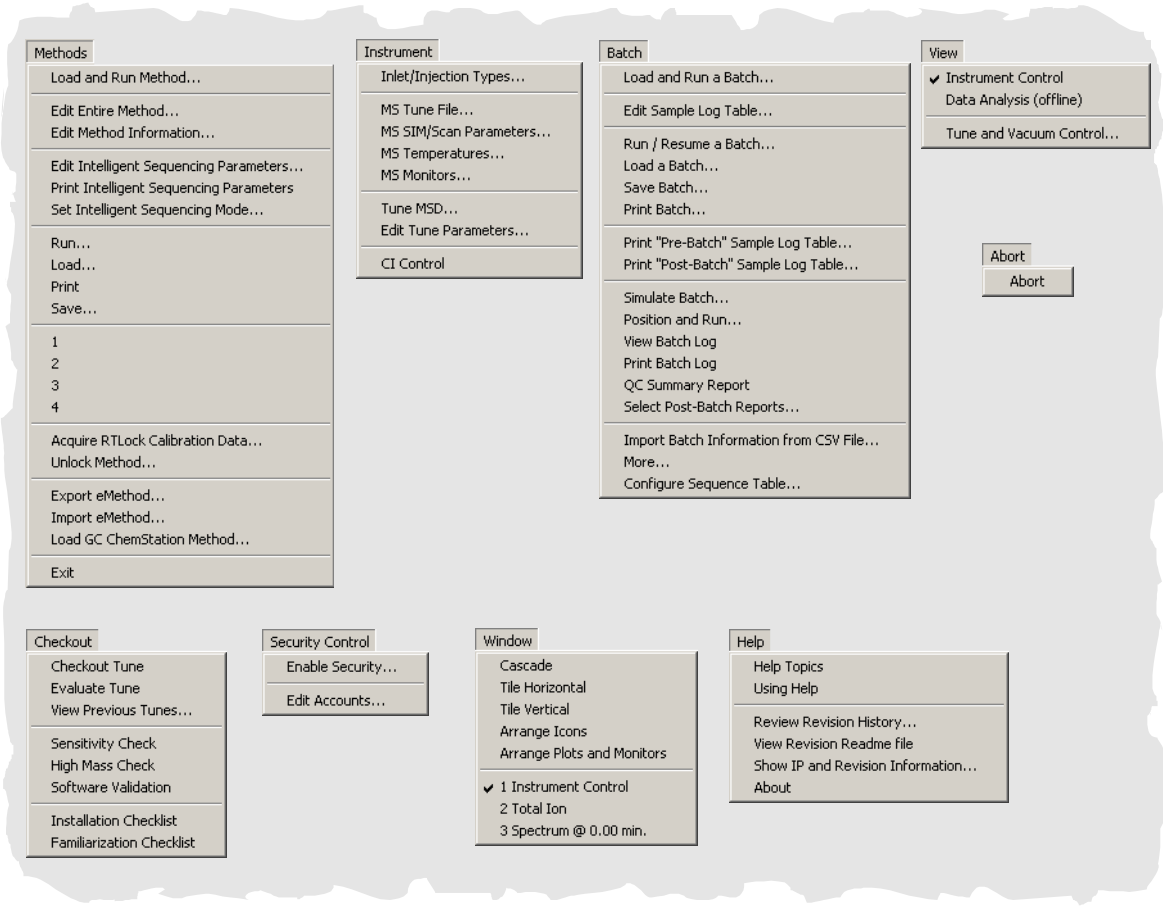
Tune Parameters
Lets you tune the MSD.



Monitor
Each monitor displays one instrument parameter. See the online help for a description of the instrument monitors.

Instrument Control Software Menus

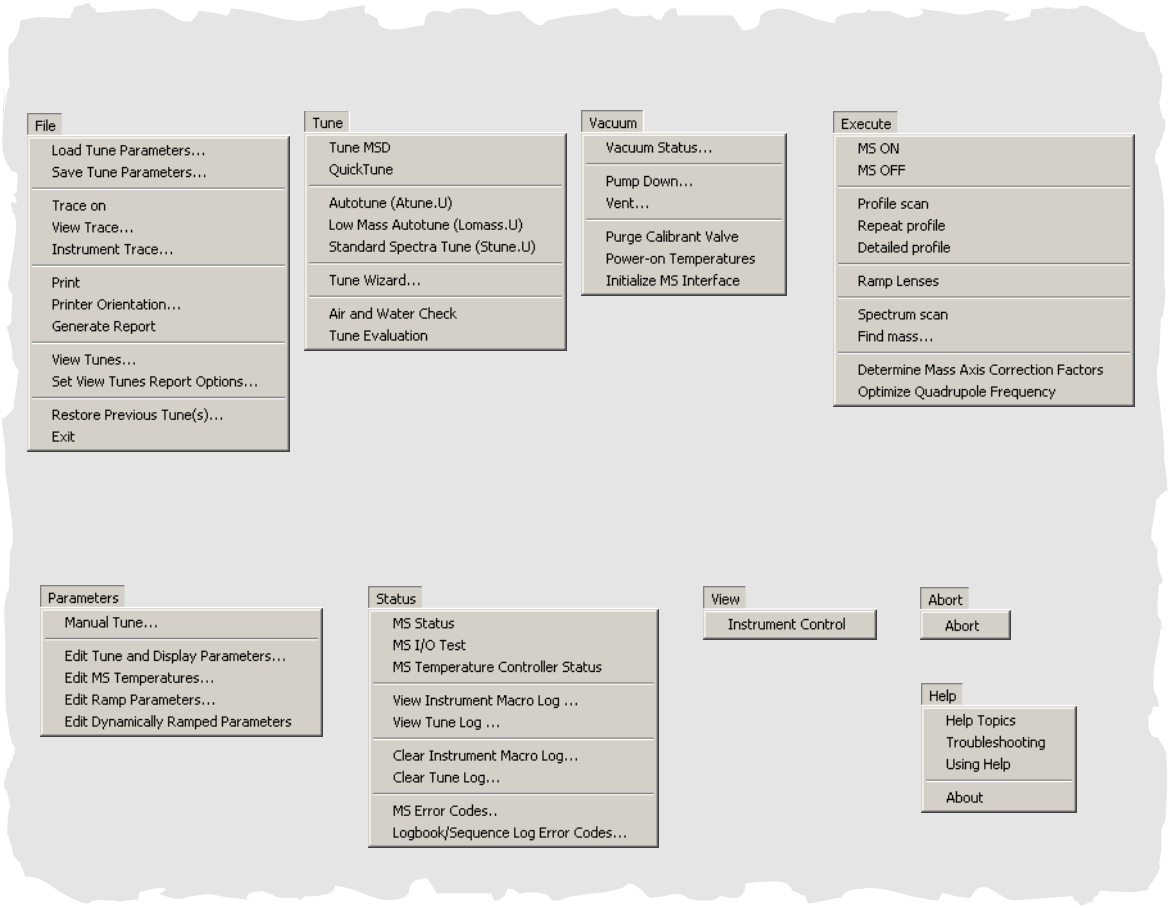
Select **View/Instrument Control** to access these menus.



NOTE The software menus shown in this book are for an GC/MSD ChemStation in Drug Analysis Mode.

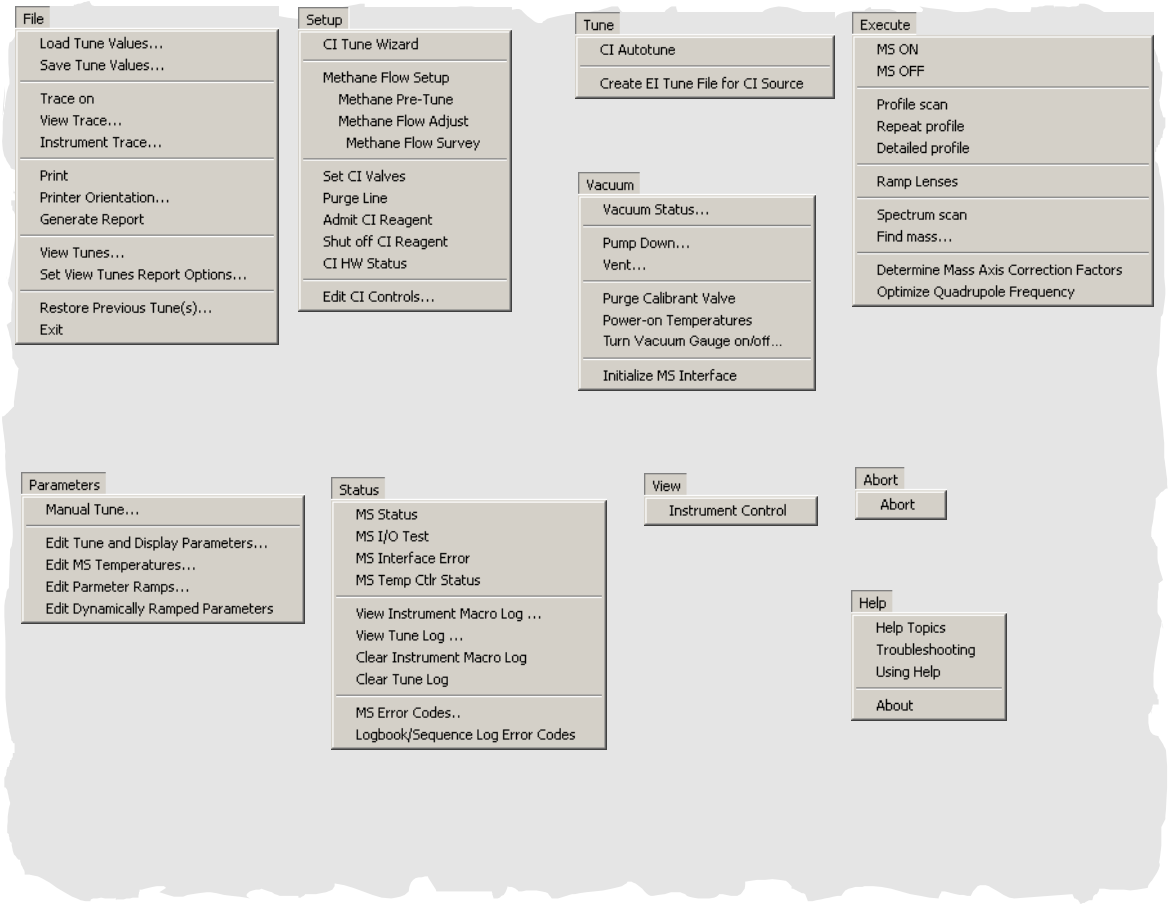
Tune and Vacuum Control Menus - EI Mode Software Menus

Select **View/Tune and Vacuum Control** from Instrument Control to access these menus.



Tune and Vacuum Control Menus - CI Mode Software Menus

Select **View/Tune and Vacuum Control** from Instrument Control to access these menus.

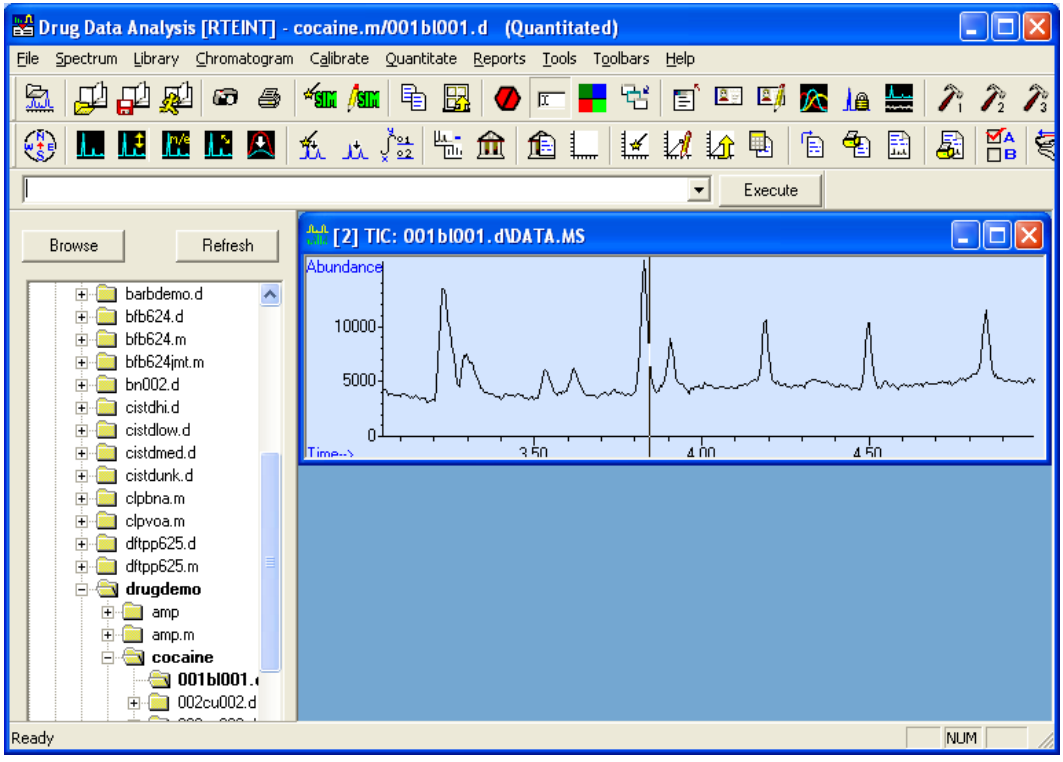


Drug Data Analysis View

The Data Analysis view is displayed when you start a data analysis instrument session or by selecting **View/Data Analysis (offline)** from the Instrument Control view. Use the Data Analysis view to perform tasks such as:

- Setting up integration parameters
- Calibrating a method
- Quantitating data
- Customizing and printing reports

NOTE See the online help for more details on the menus, buttons, or windows used in the software.



Data Analysis Toolbar Buttons

Load Data File
Loads the selected data (.D) file and displays the total ion chromatogram (TIC) for that file.

Load Method
Loads the selected method (*.M) file.

Save Method
Saves any changes made to the current method.

Run Method
Carries out only the Data Analysis portion of the current method. You must choose an output file name to print. This output file will specify the name of the file that will store the document. The document is stored in a format readable by the printer, not the program you are using to print.

Take Snapshot
Displays the data that has been acquired up to the time the snapshot is activated. GC/MS data only.

Print
Lets you print the selected window, the TIC and spectrum, or the current method.

Generate AutoSIM Method
Opens the AutoSIM Setup dialog box.

Edit SIM Parameters
Lets you edit the SIM parameters in the SIM Group Table.

Copy Window
Lets you copy the selected window to the clipboard.

Reset Windows
Rearranges the graphics windows to their default positions.

Abort
Stops a command or macro.

Toggle Command Line
Turns the display of the command line on or off.

Edit Colors
Lets you adjust the colors of various display items in Data Analysis.

Toggle Graphics
Lets you minimize or maximize the displayed graphics windows.

Close Screen Reports
Closes the currently displayed screen reports.

DrugID
Enters the DrugID mode where you can update expected retention times in an existing quantitation database on a compound-by-compound basis.

DQEdit
Enters the DQEdit mode where you can review and edit quantitation results.

Review Peak Purity
Helps you detect overlapping peaks (multiple-component peaks) in your chromatogram.

Retention Time Lock
Accesses the RTLock Setup view which is used for retention time locking tasks.

Generate Signal-to-Noise Report
Lets you perform a signal-to-noise check and then display or print the report.

CUSTOM TOOL 1
Lets you run a user-created macro. This macro must first be created and then named CUSTOMTOOL1. See the online help for Using and Writing Macros, and Data Analysis Commands.

CUSTOM TOOL 2
Lets you run a user-created macro. This macro must first be created and then named CUSTOMTOOL2. See the online help for Using and Writing Macros, and Data Analysis Commands.

CUSTOM TOOL 3
Lets you run a user-created macro. This macro must first be created and then named CUSTOMTOOL3. See the online help for Using and Writing Macros, and Data Analysis Commands.

CUSTOM TOOL 4
Lets you run a user-created macro. This macro must first be created and then named CUSTOMTOOL4. See the online help for Using and Writing Macros, and Data Analysis Commands.

CUSTOM TOOL 5
Lets you run a user-created macro. This macro must first be created and then named CUSTOMTOOL5. See the online help for Using and Writing Macros, and Data Analysis Commands.

Hide/Show Navigation
Toggle icon that lets you show or hide the Explorer pane.

Draw Chromatogram
Redraws the original chromatogram of the current data file without labels or integration marks.

Scale Chromatogram
Not available in Drug Analysis mode.

Extract Ion Chromatograms
Extracts and displays extracted ion chromatograms (EICs) from the TIC of the current data file.

Merged Format
Causes EICs to be displayed overlaid on each other.

Overlay Chromatograms
Not available in Drug Analysis mode.

AutoIntegrate
Tries to find the best integration parameters for the current chromatogram and then integrates the chromatogram.

Integrate
Integrates the current chromatogram using parameters set for the current integrator.

Integration Parameters
Opens a dialog box for editing current integrator's parameters or events.

Subtract Spectrum
Subtracts one spectrum from another and displays the difference.

Select Library
Lets you select the libraries that will be used for PBM searches of the currently selected spectrum.

Library Search Report
Integrates the current TIC, searches the current library, and generates a report.

Set Up Quantitation
Lets you set up a quantitation database by specifying quantitation database globals and entering compounds in the database.

AutoQuant Setup
Provides a semi-automated way to create a quantitation database.

Edit Compounds
Lets you review and edit information in the quantitation database compound-by-compound.

Update Calibration
Lets you add, delete or update a calibration level in the current quantitation database.

Calculate Quant Report
Quantitates the current file and generates a quantitation report.

Generate Quant Report
Generates a quantitation report for a file that has already been quantitated.

Print Quant Report
Prints the quantitation report.

Custom Reports
Starts the Custom Reports software. If the method does not have a quantitation database, or no data file is loaded, you can use default values.

Print Custom Report
Prints the custom report template specified by the method, using the current data file.

Data Analysis Options
Opens the Select DA Options dialog box.

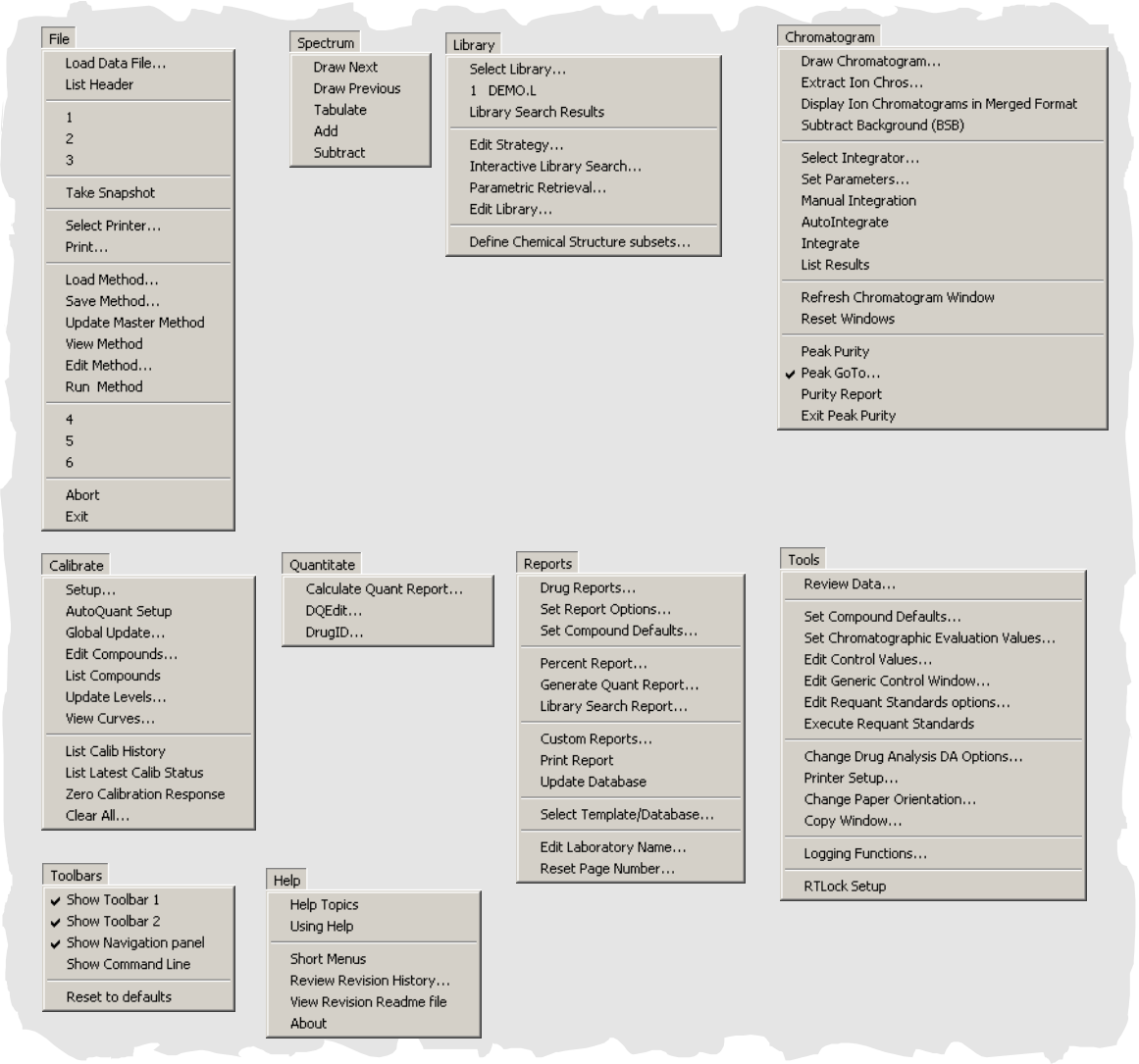
Switch Data Analysis Mouse Actions
Toggles the right-click functionality of the mouse from traditional actions to the new right-click menu options.

Show/Hide Stack (Variable Watch)
Lets you choose to show or hide the stack (variable watch) window.

Online Help
Displays the GC/MSD ChemStation online help.

Drug Data Analysis Software Menus

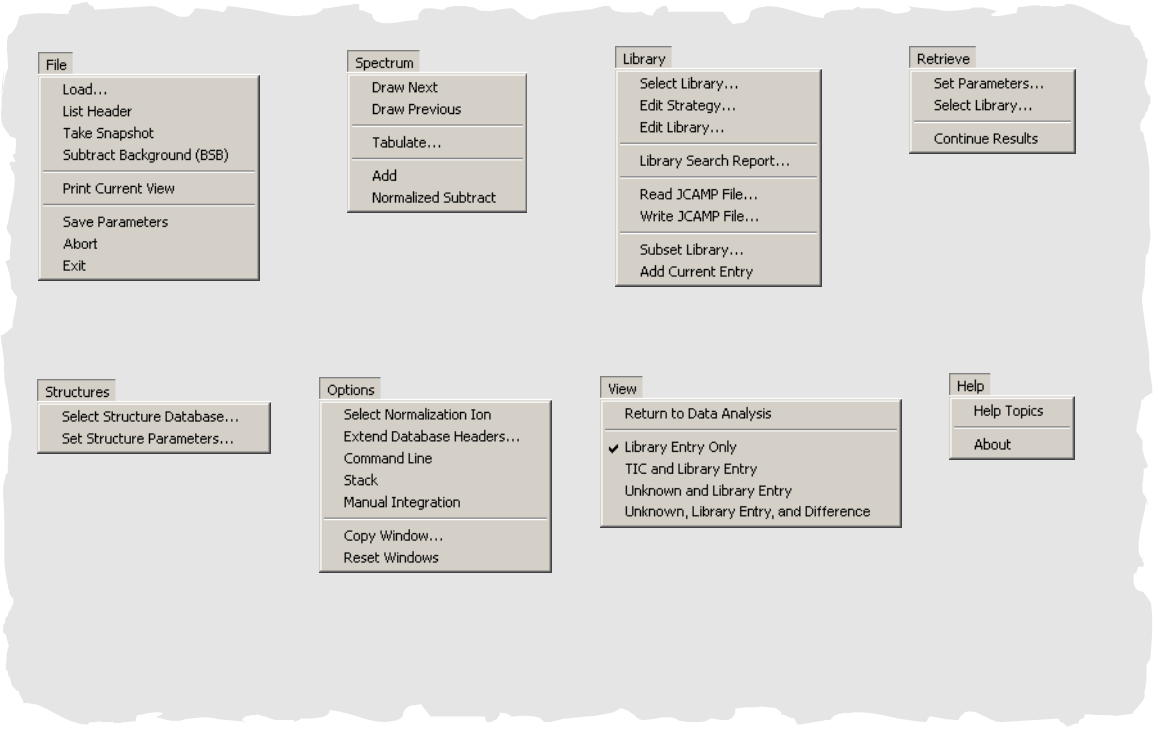
Select **View/Data Analysis (offline)** to access these menus.



The View menu in Data Analysis lets you access other utilities within Data Analysis (shown on the following pages).

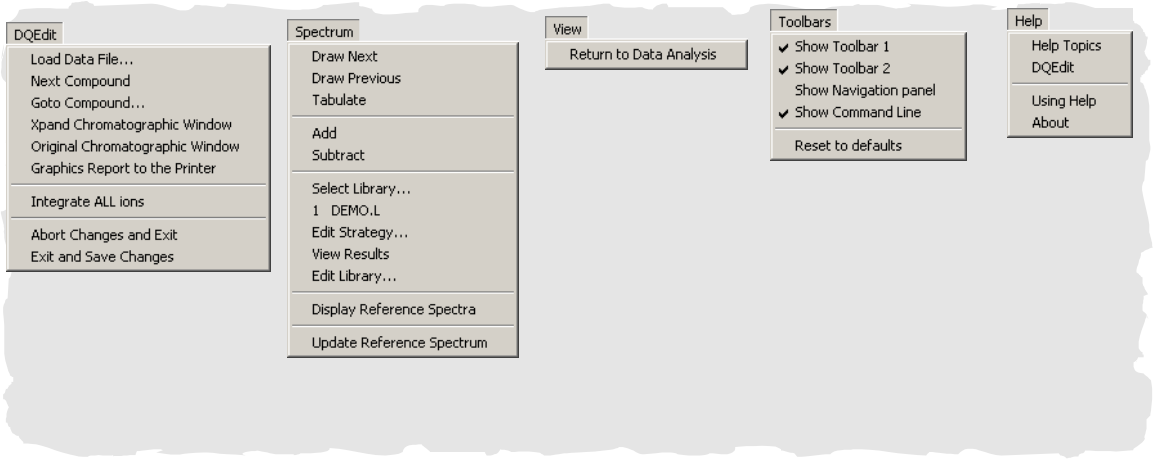
Parametric Retrieval Software Menus

Select **Library/Parametric Retrieval** in Drug Data Analysis to access these menus.



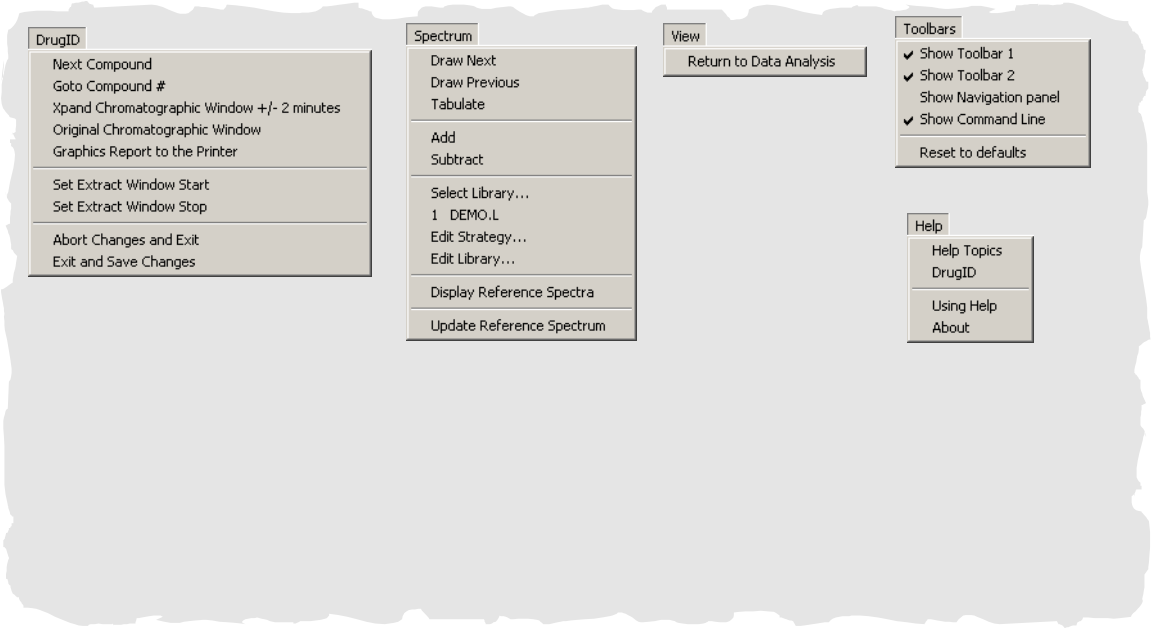
DQEdit Software Menus

Select **Quantitate/DQEdit** in Drug Data Analysis to access these menus.



DrugID Software Menus

Select **Quantitate/DrugID** in Drug Data Analysis to access these menus.

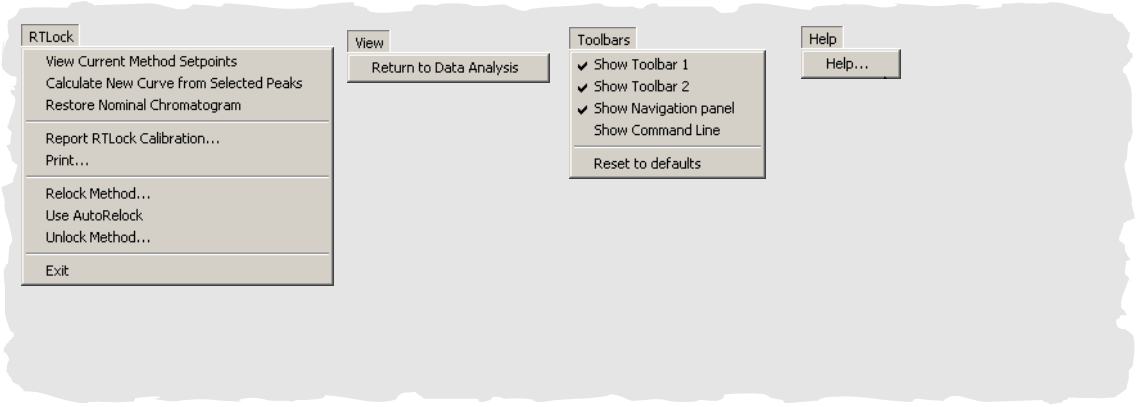


Review Data Software Menus

Select **Tools/Review Data** in Drug Data Analysis to access these menus.

RTLock Software Menus

Select **Tools/RTLock Setup** in Drug Data Analysis to access these menus.



To Tune your MSD

You should tune the MSD periodically to maintain its optimum performance. Tuning is the process of adjusting MSD parameters so the instrument meets certain performance criteria. How often you should tune is determined by the number and type of samples you are running, as well as the overall condition of your system.

NOTE Always tune the MSD with the same GC oven temperature and column flow, and the same analyzer temperature that will be used for data acquisition.

Keep the Tune reports in a notebook so that successive reports can be easily compared.

To tune the MSD

From the Instrument Control view:

1 Select **View/Tune and Vacuum Control**.

2 From the Tune menu select one of the following, depending on the instrument performance required by your application.

- **Tune MSD**
  Results in maximum sensitivity over the full scan range.
- **QuickTune**
  Adjusts the peak width, mass assignment, and abundance without changing ion ratios.
- **Autotune (Atune.U)**
  Tunes for maximum response over full scan range.
- **Low Mass Autotune (Lomass.U)**
  Tunes for the low-mass range.
- **Standard Spectra Tune (Stune.U)**
  Results in a standard response over the full scan range. This option may reduce sensitivity.

- **Tune Wizard. . .**
  Displays a series of dialog boxes that let you set abundance ratio targets and adjust tuning criteria. This is used for target tuning.
- **Air and Water Check**
  Generates a standardized measurement and report of the system air (nitrogen *m/z* 28) and water (*m/z* 18) levels relative to PFTBA mass 69. Use this item to check for leaks. The abundance of *m/z* 28 should be less than that of *m/z* 18, and each should be less than 5% of *m/z* 69.
- **Tune Evaluation**
  Evaluates the current tune file.

3 Review the Tune report.

4 To view the history of tune results, select **File/View Tunes**.

To use manual tune

Manual tuning lets you interactively set the MSD parameters, such as lens voltages and tuning masses, to values that meet the needs of your particular analysis. Using manual tuning you can often obtain greater sensitivity than you can with autotune.

Manual tuning allows you to ramp individual parameters and to specify the range and step size for the ramp. The results of the ramp are displayed visually with the optimum value for the parameter clearly marked on the plot.

You can acquire two types of data in manual tune: profile scans (plots the abundance and peak shape of the tune masses) and spectrum scans (scans plot response across the entire mass range).

See the online help for more details about manual tuning.

To Run a Sample

To set up the GC for use with the MSD

From the Instrument Control view:

1. Select **Instrument/Inlet/Injection Types**. Select the appropriate injection source and select the **Use MS** checkbox. Click **OK**.
2. Select **Methods/Run**.
3. When the Start Run box appears, specify the sample information:
   - ❒ Specify a unique data file name for the sample.
   - ❒ Enter the position number of the sample vial in the **Vial** field (1–100).
   - ❒ (Optional) Fill in the **Operator Name**, **Sample Name**, and **Misc Info** fields to document the injection.
   - ❒ Make sure that the *Data Acquisition* option is selected. Select the **Data Analysis** option if you want to generate any of the reports specified in the method.
4. Click **Run Method**.
5. When the Please select a sample type dialog box appears, select the appropriate sample type and click **OK** to initiate the run.

CAUTION Do *not* use **Start** on the GC to start a run when using the autosampler.

To inject a sample manually

In the Instrument Control view:

1. Select **Instrument/Inlet/Injector Types**.
2. In the Inlet and Injection Parameters dialog box, select **Manual** as the injection source. Click **OK**.
3. On the GC keypad, press [Prep Run]. This cancels the gas saver flow, brings the inlet flow to its setpoint value, and closes the purge valve (for splitless injection only).
4. Select **Methods/Run...**

5 When the Start Run box appears, specify the sample information as described below:

- ❒ Specify a unique data file name for the sample.
- ❒ (Optional) Fill in the **Operator Name**, **Sample Name**, and **Misc Info** fields to document the injection.
- ❒ Make sure that the *Data Acquisition* option is selected.
- ❒ (Optional) Select the **Data Analysis** option if you want to generate any Data Analysis reports specified in the method.

6 Click **Run Method**.

7 When the Please select a sample type dialog box appears, select the appropriate sample type and click **OK** to initiate the run. If the temperatures are stable, the Prepare To Inject box appears. Otherwise, the message **Waiting for GC ready** is displayed.

8 When the GC temperatures have stabilized (6890 GC - the Pre Run light on the GC is steady, 6850 GC - the Not Ready light is off), inject the sample and press [Start] on the GC.

CAUTION Do not inject before the GC is ready. This will cause inconsistent results.

See also "To set up a batch (sequence)" on page 39.

To Edit a Method

You can use the predefined method files for amphetamines, cocaine, opiates, PCP, and THC as templates for your own analyses. The predefined methods (*.M) files are found in the folder **msdchem/msdemo/drugdemo**.

To use these methods, you will need to edit the method parameters based on your particular column, instrument, and analysis requirements.

To edit a method

1 Select **Methods/Edit Entire Method**. The Check Method Sections to Edit box is displayed.

2 Select the method sections (shown below) you want to edit. A series of boxes will then prompt you for changes specific to the parts you selected:

- **Method Information**
  Lets you modify information about the method, save a copy of the method with the data file, and specify sections of the method to run.
- **Intelligent Sequencing**
  Lets you specify all the Intelligent Sequencing criteria for Batch Intelligent Sequencing prior to running a batch.
- **Instrument/Acquisition**
  Lets you modify instrument setup, tuning, and acquisition parameters in the current method.
- **Data Analysis**
  Lets you edit the Data Analysis portion of the method (report type, defaults for internal standards and compounds).
- **Requant Standards**
  Lets you specify the requant standards options to use to requantitate calibrators once the calibration table is complete.

3 When you have finished editing the selected method sections, you are prompted for the following information:

- To save the method. Save your current method or enter a new file name.
- Whether to turn Intelligent Sequencing on or off for this method.
- What to do if the batch aborts

Batch Intelligent Sequencing

Intelligent Sequencing is a feature of DrugQuant that makes "programmed" decisions based on the quantitative and qualitative results of sample analysis. The "programming" is done by editing the Intelligent Sequencing portion of a method to set up the Intelligent Sequencing parameters and then setting up a batch analysis using the Sample Log Table.

Intelligent Sequencing increases the number of batches that may be run without operator intervention. This is accomplished by saving a method with preset decisions that are carried out depending on the result of each sample.

One example of an Intelligent Sequencing decision is to:

> *"Inject a blank before continuing if the concentration of a specimen is found to be above the carryover limit."*

Using the above example of an intelligent sequencing decision, if a specimen is found to be above the carryover limit, a blank will be injected. Therefore, the next injection will have much less chance of being contaminated by the highly concentrated specimen since the blank injected immediately before it will have “cleaned up” the system significantly. Ordinarily, this same decision would have been made by the instrument operator, who would need to examine every injection as it is occurring.

To Set up Batches (Sequences)

To use Intelligent Sequencing

Use the procedure below to set the Intelligent Sequencing options you want and identify the batch to which it applies.

1. Select **Methods/Load** to load the method you want to use for Intelligent Sequencing.
2. Select **Methods/Edit Entire Method**.
3. Select the **Intelligent Sequencing** option and click **OK**.
4. When the Intelligent Sequencing Parameters dialog box appears, set the desired options for Blanks, Negatives, Controls, and Specimens sample types.

   Blanks: Blank samples contain solvent only (no sample matrix or ISTD). They do not need to be interspersed between samples, but rather can be grouped together in the autosampler tray. Specify the starting vial number for the blanks, the number of blank vials, how often to inject a blank, and maximum number of reinjections.

   Negatives: Negatives are clean sample matrix that ISTD has been added to.

   Controls: Controls are samples spiked with both ISTD and analytes to check the analytical procedure.

   Specimens: Specimen samples are the unknowns you are analyzing for the compounds of interest. Select the action to take when the concentration of analyte exceeds the carryover level.
5. Click **OK** when the desired options have been set. Be sure to save the method when prompted; otherwise the changes will be lost.
6. When prompted, select to turn on (or leave on) Intelligent Sequencing and click **OK**.
7. You are now asked what to do if the batch pauses. Make your selection and click **OK**.

Now, you are ready to set up the batch.

To set up a batch (sequence)

A batch allows you to automate the analysis of one or more samples. When an ALS is installed, the entire analysis, from injection of the sample through reporting of results, can be automated using the Batch Intelligent Sequencing software. *See the online help for more information.*

1. If the Sample Log Table is not already open, select **Batch/Edit Sample Log Table**.
2. Click on a blank line in the **Type** column of the table. Then click the drop-down arrow and select the type of sample you are going to run.
3. Use the Tab key or the mouse to move to the **Vial** box and enter the vial number.
4. Move to **Method/Keyword** and enter the name of the method to be used for the current sample. (For a list of methods, click the **?** button.)
5. Supply the **Data File** name, a **Sample Name**, any **Comment** and the **Expected Bar code** (if you have a bar code reader).
6. Move to any other fields that apply to your sample.

   The right mouse button displays a menu enabling you to copy, cut, paste, fill, insert, and repeat table data. See online help for more information.
7. When you are finished, click **OK**.
8. Select **Batch/Save Batch**, specify a name and click **OK**.

To Analyze MS Data

To load a data file

To load the data file in Drug Data Analysis:

1 Select **File/Load Data File**.

2 Select a data file name and click **OK**. The chromatogram for the data file is loaded and displayed in window [2].

CAUTION A data file must be loaded to perform any of the tasks in this section.

To integrate a chromatogram

1 If the integrator you wish to use is not currently selected, click **Chromatogram/Select Integrator**. Choose an integrator and click **OK**.

2 Select **Chromatogram/Integrate**.

3 (Optional) Select **Chromatogram/List Results**. A report of tabulated results is displayed on the screen. When you are finished viewing the results, click **Close**.

To quantitate a date file

1 Select **Quantitate/Calculate Quant Report**.

2 Select the options you want to use and click **OK**.

To select a spectrum

Double-click the *right* mouse button on the time point of interest in the chromatogram. The spectrum appears in window [1].

To zoom in

1. Position the pointer at one corner of the area you wish to expand in a chromatogram or spectrum.
2. Press and hold the *left* mouse button while dragging the mouse to select the area you wish to expand.
3. Release the mouse button. The selected area expands to fill the existing window.

To zoom out

1. Position the mouse anywhere in the zoomed window.
2. Double-click the *left* mouse button.

To average spectra

1. Position the pointer in the chromatogram at the starting time for the range you want to average.
2. Press the *right* mouse button while dragging the mouse to the end of the range you want to average.
3. Release the mouse button. The spectra in the selected range are averaged and the averaged spectrum is displayed in window [1].

To add two spectra

1. Select a spectrum (double-click the *right* mouse button in the chromatogram).
2. Select a second spectrum (double-click the *right* mouse button in the chromatogram).
3. Select **Spectrum/Add**. The two spectra are added together and the resulting spectrum is displayed in window [1].

To subtract two spectra

1 Select a spectrum (double-click the *right* mouse button in the chromatogram).

2 Select the spectrum to be subtracted (double-click the *right* mouse button in the chromatogram).

3 Select **Spectrum/Subtract**.

The spectrum selected in step 2 is subtracted from the spectrum selected in step 1 and the resulting spectrum is displayed in window [1].

To subtract background spectra

1 Select a spectrum or average a range of spectra to subtract from the data file.

2 Select **Chromatogram/Subtract Background (BSB)**. The system performs the following tasks:

- The selected spectrum is subtracted from every scan in the current data file.
- The subtracted data is stored in a BSB subdirectory in the same directory as the data file.
- The subtracted data file becomes the current data file and is displayed in window [2].

To Use Spectral Libraries

To integrate and search peaks

Use the following procedure to integrate a TIC and automatically generate a library search report for each peak detected.

1 In Drug Data Analysis, load a data file. The TIC is displayed.

2 Select **Reports/Library Search Report**.

3 When the **Library Search Report Options** dialog box appears, select the options you want for the library search report:

- Select either **Summary** or **Detailed** to determine the report format.
- Select one or more destinations (**Screen**, **Printer**, and **File**).
- Select an **Integration Parameter File** (leave the field blank to autointegrate using the GC/MSD ChemStation integrator).
- Select which spectrum from each peak to use (**Apex, Apex - Start of Peak, Apex - Background at time, or Peak Average**).

4 Click **OK** to initiate the search.

The chromatogram is integrated and a spectrum from each peak is searched. The results of the integration appear on the screen. The library search report is sent to the destinations selected in step 3.

5 Select **Chromatogram/List Results** to view the tabulated integration results.

To search a single spectrum

1 In Data Analysis, load a data file. The TIC is displayed.

2 Select a **spectrum**. The selected spectrum appears in a window below the chromatogram.

3 Initiate the library search by double-clicking the right mouse button in the window containing the spectrum.

When the search is complete, the search results appear on the screen. The spectrum for the unknown, the reference spectrum you select from the list of hits, and, if available, the chemical structure of the reference compound is displayed.

4 To view other spectral data:

- Click another compound in the hit list to display a different reference spectrum.
- Select the **Difference** checkbox to display the difference between the unknown and the reference spectra.

5 To view other information:

- Click **Statistics** to display information about the quality of each hit found in the list.
- Click **Text** to view the header information stored in the library for the current reference spectrum.

6 Click **Print** to print a copy of the displayed spectra.

7 Click **Done** to clear the library search results from the screen.

To Use Review Data

Review Data is an interactive tool used to search for specific drugs. When you click on **Tools/Review Data**, you are prompted to select a drug class. For example, you might select **amphetamines** as your drug class, and choose the method **AMP.M**. The Review Data window is displayed with the selected drug class name (in this case, Amphetamines) in the menu bar. When you open this drug class menu, various data review items are available.

To review a single data file

When you select **Review Single Data File**, you will be prompted to load a data file and the compound you want to search. A window divided into three parts is displayed:

- The left half of the window displays the ion traces overlaid or separated, depending on how the Data Analysis defaults have been set.
- The upper right window displays a screen that varies according to acquisition mode:
  - **Scan** displays the unknown spectrum and the first hit of the library search.
  - **SIM** displays the TIC window centered on the peak of interest.
- The lower right window displays quantitative data about the drug or compound of interest.
  - ❒ To move to the next compound in the Quant database, double-click the right mouse button in the text window.
  - ❒ To move to the previous compound in the Quant database, double-click the left mouse button in the text window.

To review multiple data files

You can review multiple data files in a batch. When you select **Review Multiple Data Files**, you will be prompted to load the first file in a batch. Then you will be prompted to select a file type, such as **Specimens** or **Blanks**.

- To move to the next file in the batch that matches the file type selected, double-click the right mouse button in the ion window.
- To move to the previous file in the batch that matches the file type selected, double-click the left mouse button in the ion window.
- To select a specific compound to be searched for in the selected file type, double-click the left or right button in the text window.

Autobrowsing multiple files

If you select **Tools/Review Data/AutoBrowse Multiple Data Files**, the autobrowsing time is set and the system will automatically advance through all of the data files in a batch. You are asked to specify the first file of the batch.

- To modify the length of time the system displays each file, select **Tools/Change Drug DA Options/Change Autobrowse Time**.

To Update Retention Times Using DrugID

DrugID is a tool in Drug Data Analysis that is used for updating the retention times and qualifier ion ratios in a quantitation database on a compound-by-compound basis. To shift the retention times of all compounds at once, use **Calibrate/Global Update**.

Before going to DrugID, load a calibration sample data file that was acquired under the conditions that are to be used for all subsequent target compound analyses for the method you are using.

1. With the desired method loaded, load a data file for a calibration standard using **File/Load Data File**. The method must have an established quantitation database.
2. Select **Quantitate/DrugID**. The menu bar changes and chromatogram, spectrum, and text windows are displayed.
3. Click and drag the right mouse button to define a baseline for a peak in the displayed chromatogram. Since DrugID uses integrated retention times, you don't need to draw the baseline precisely at the beginning and ending of the peak. In most cases you can draw the baseline below the actual data, in the area of the axis.

   The retention time of the integrated peak will be the new expected retention time for the compound. The beginning and ending times for extracting the signal will move relative to the new expected retention time. If no integration has been performed on the currently selected compound, then the calibration table is not modified.
4. To process the next compound, double-click the right mouse button anywhere in the text window in the lower right corner of the screen or select **DrugID/Next Compound**. If you performed a manual integration, but it was incorrect and you wish to proceed without updating that compound, first select **DrugID/Original Chromatographic Window** from the menu. This will restore the chromatogram to its original state and remove any integrations.

If the compound you are updating is an internal standard, a prompt appears to **Update relative target compounds?** If yes is selected, the target compounds associated with this internal standard have their expected retention times updated to maintain their relative retention times with the internal standard. After this update, the next internal standard is displayed.

5 Select **DrugID/Exit and Save Changes** to exit the DrugID mode and to save the changes you have made.

6 To verify updated retention times, select **Quantitate/Calculate Quant Report** in the Drug Data Analysis window. When prompted, specify a summary report to the screen, then click **OK**. Review the displayed report to see that all compounds were found.

To Review Quantitative Results Using DQEdit

DQEdit is a tool in Drug Data Analysis that lets you review and edit quantitation results once a data file has been quantitated. It lets you manually integrate peaks and update the quantitative results as needed.

1. With the desired method loaded, load the data file of interest using **File/Load Data File**.
2. Select **Quantitate/DQEdit**. This item is available only if the current data file has been quantitated with the current method.

   A new set of menus is loaded and the quantitative results for the current data file are copied into a temporary file. In DQEdit mode, all changes are made to this temporary file. The original data is only overwritten if you save the changes as described in Step 5 or 6 below.
3. To manually integrate a peak, draw a new integration baseline by clicking and dragging with the right mouse button.

   Select **DQEdit/Integrate All ions** to access all the ions; otherwise only the target ion is displayed.

   If the peak of interest is on the edge of the window, double-click the left mouse button in the chromatogram window or select **DQEdit/Xpand Chromatographic Window** ± 2 minutes.
4. To go to the next compound, double-click the right mouse button in the text window or select **DQEdit/Next Compound**.
5. (Optional) To load a different data file, select **DQEdit/Load Data File**.

   You will be prompted to save the changes to the current data file before the new file is loaded. Repeat steps 3–5 to evaluate the new data file.
6. Select **DQEdit/Exit and Save Changes** and select **Yes** to save the changes to the current data file and to exit DQEdit.

Use **DQEdit/Abort Changes and Exit** only if you want to exit without saving the changes.

The fact that you have used DQEdit to modify results will be noted when you print a drug report for a compound that has been changed. The note on the report states that manual integration was used (to determine the area response for the compound).

To Use Retention Time Locking

Retention time locking (RTL) is a procedure that evaluates characteristics of a particular method (column, flow setpoints, oven parameters) so that any changes to the column, which would normally impact retention times, are negated. The procedure involves collecting data for a compound (whose desired retention time is known) at various inlet pressures around the current method setpoint (–20%, –10%, nominal, +10%, +20%). The five resultant runs are then evaluated and a pressure/retention time curve is generated to characterize that particular instrument. From the curve, a predicted pressure which causes the lock compound to elute at the desired time can be calculated and stored so that the method will run at that pressure.

To lock an MS method

1. From Instrument Control, load the method you want to lock. Edit the method parameters, if necessary.
2. For ALS injections, put the vial in position 1.
3. Select **Instrument/Acquire RTLock Calibration Data**. This initiates the collection of the RTL calibration files.

   The nominal pressure will be evaluated for the calibration range of –20%, –10%, +10% and +20%, and five runs will be made automatically. You are prompted that the five runs will be made, and if any previous calibration data exists, you are alerted to this fact as well. The five data files will be stored in the method directory under a folder named RTLOCK with the data file names of RTLOCK1 - RTLOCK5.
4. Following data collection, a new session of Data Analysis will be initiated, and the nominal run (RTLOCK3.D) will be loaded. Select the peak (click and drag right mouse button) you want to use for RTL calibration calculations.
5. The spectrum of the selected peak will be displayed. Click **Yes** to have the software automatically locate the lock compound peak in the remaining four runs. The software will

now perform spectral comparisons and curve fit determinations. The five selected peaks are then displayed.

6 The curve equation (based on the retention time vs. pressure values) is displayed and you are asked if you want to continue. Click **Yes**.

7 Next, enter the lock retention time you want to use and click **OK**.

8 Click **Yes** to save the lock pressure information to the method. Enter the lock compound name you want to use and click **OK**.

9 You are now given the option to delete the calibration data files (RTLOCK1.D - RTLOCK5.D). Select **Yes** or **No**. The method is now locked.

Whenever a locked method is loaded into Instrument Control, the title bar will indicate that the method is locked, and which compound was used for the lock. The pressure (online instruments only) will be set to the locked pressure.

NOTE When a locked method is run, the pressure is restored to the locked pressure value EVEN if you have made changes using the GC keypad or from Instrument Control.

© Agilent Technologies, Inc.

Printed in USA, June 2005

G1701-90055